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# SEED MYCOFLORA OF SOYBEAN VARIETIES AND FIELD RESISTANCE EVALUATION TO SOYBEAN RUST

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***ABSTRACT***

***Mycoflora associated with soybean seeds of three varieties collected from IRAD of Garoua and their field resistance to soybean rust,were studied. Fungal profile of soybean seeds was carried out in the Laboratory of Plant Pathology, Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon. The field study was conducted during cropping season in the Polyvalent Station of Institute of Agronomic Research for Development (IRAD) based in Foumbot. Overallmore than eight fungi were isolated by using blotter and agar technique respectively. According to both methods, fungi which were frequently isolated were Aspergillusniger, Aspergillusflavus, Fusariumoxysporumand Rhizopusstolonifer. However, fungi such as Macrophominaphaseolina, Fusariumculmoreum, Verticillium, Trichoderma were also present. Plant height varied from54.21 to 61.68 cm according to the variety TGX 1835-10E produced the tallest plants follow by EMGOPA301 (57.35 cm) and GOINA (54.21 cm). Average grain yields ranged from 802.2 to 4010.05 kg/ha. Variety TGX1835-10E produced the highest grain yields while GOINA and EMGOPA301 had the least. Rust severity varied from 16.13 (TGX-1835-10E) to 60.55% (EMGOPA301) during the reproductive phase. In maturity, Emgopa 301 and Goinia varieties have reached 100% severity while the TGX-1835-10E variety presented 34.30% severity. Data obtained in this study revealed that rust infection is serious problem in the study area.***

***KEYWORDS: Soybean, Fungal Profile, Varietal Evaluation, Rust Infection, Yield***

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## INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is an important species of legume native to East Asia. It is an annual plant, a worldwide economic crop and the most important cultivated legume with hundreds of food, feed and industrial uses. Fat free (defatted), soybean meal is a primary and relatively low cost source of protein for animal feeds.Soya vegetable oil is another valuable product of processing the soybean crop (Ramesh et al, 2013). In addition soybean is an excellent source of major nutrients, about 40% of dry matter is protein and 20% fat (Cald Well, 1973). It is one of the most important sources of oil and proteinin the world and it is commonly used in both human and animal diets (Onwueme and Sinha, 1991; Ariyo 1995). Also, soybean is one of the major crops on which government and international aid can focus on because it can produce the highest yield of protein per unit of land area of any plant or animal food source while at the same time producing calories (Singh et al, 1989). Soybean (*Glycine max* (L.) Merill.) is not an indigenous crop in Cameroon, it is however, gaining popularityin the country because of its numerous potentials thatrank it even better than cowpea in the supply of highquality protein.

In Cameroon, it is used in nutrition of infants, patients and old persons. Since 2008, many varieties from various origins were introduced in Cameroon by research carried out in the sudano-sahelian zone of Cameroon by SODECOTON (*Société de Développement de Coton*) in the aim of farming diversification to meet-up the fall in cotton production. As new varieties of soybeans are continuously being developed to meet demand, it is important to evaluate these new varieties in the West of Cameroon where the bulk of it is produced commercially.

As soybean acreage has expanded throughout the world, diseases have increased in number and severity. All parts of the soybean plant are susceptible to a number of pathogens which reduce the quality and/or quantity of seed yield.Among diseases that threaten this crop, soybean rust is one of the most severe (Hartman et al., 1999). Favorableenvironmental conditions for disease development result in substantial yield losses (Hartman et al., 1991; Yorinori et al.,2005). The causal fungus *Phakopsorapachyrhizi*Sydow was documented in 1903 as *Uredo sojae* from rust-infected leaves of*G. max* subsp. *soja* or wild soybean in Japan (Hennings, 1903), and has since been reported in many countries throughout the Eastern Hemisphere (Hartman et al., 1999). It has since spread to other parts of the world. It is a destructive soybean disease in humid tropical and sub-tropical regions (Tichagwa, 1999). It can cause pre-mature leaf fall and reduce oil percentage, recovery from grains, from 42.7 to 14.94% and yield loss between 10 - 100% (Yujun.et al., 1999). It is therefore, important to evaluate newly developed soybean varieties for their susceptibility to this important disease.

The objectives of this work therefore were to evaluate the performance of a set of newly developed soybean varieties atFoumbot in the West region of Cameroon for grain yield and their reactions to rust infection

## MATERIAL AND METHODS

### Location of the Study

The field study was conducted in the 2015 cropping season in the Polyvalent Station of the Institute of Agricultural Research for Development based in Foumbot, located in Noun Department, Region of western Cameroon. The altitude of IARD is 1010.5m. The latitude and longitude for this station are 5°28 N and 10°33 E (Ndo, 2011). Soils are volcanic, mean annual rainfall amounted to 1538.8 mm and more rainy periods of the year are from July to September. The temperature fluctuates between 20 and 24 ° C. The evaluation of sanitary quality of soybean seeds was carried out during March and April 2015 in the Department of Plant Protection, Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon.

### Seed Samples and Experimental Design

Soybean seed samples were collected from IARD of Garoua in the North Cameroon. That include: TGX-1835-10E, EMGOPA 301 and GOÏANIA. The plantings were done in mid-July of the year using a randomized complete block design with three replicates. Each block consisted of three plots of 3 x 3 m interspaced at 0.5 m. An alley of 1 m separated blocks. The seedlings took place 10 days after ploughing. The soybean seeds were planted at a spacing of 0,5m between rows and 0,2m within rows, giving a plant density of 300000 plants/ha. The sowing technique adopted was hill dropping with 3 seeds per stand. Seven days after germination, the seeds were thinned down to one seed per stand. No fertilizer was applied and manual weeding was done as at when due. The percentage of lift of soy beans has been evaluated up to 7 days. At maturity, agronomic data were taken from plants in two middle rows of each plot to reduce border effect. Data recorded include the rate of emergence, plant height, number of pods per plant, grain yield per hectare and the weight

of 1000 grains after drying.

**Soybean Rust Severity and Incidence Assessment**

Soybean rust severity and lesion type were assessed on fifteen marked plants randomly selected in each plot and used during growth stages. Some times after (between 2 and 3 weeks after planting), the soybean varieties were observed for natural development of symptoms of rust infection using a five-point scale disease severity ratings. Where 1 = no obvious spot on leaves, 2 = slight infection, less than20% of leaves showing symptoms, 3 = moderate infection, 20 – 40% of leaves showing symptoms, 4 = severe infection, 40 – 70%of leaves showing symptoms and 5 = leaf abscission, over 90% ofthe leaves loosing most of their photosynthetic areas.

**Seeds Health Evaluation**

200 seeds were taken from each variety, randomlyfor pathogenic fungi isolation. Agar plate method was used (by using potato dextrose agar) alsoBlotter technique. In Blotter technique, three layers of blotter paper discs were placed in sterilized Petri dishes (9 cm) and moistened with sterilized distilled water. Seeds were surface sterilized as per the ISTA recommendation (ISTA, 1966) and in each Petri plate, ten seeds were placed for isolation of mycoflorafor both methods. Plates were incubated for seven days by providing 12 hours light and 12 hours darkness alternatively at 25 ± 2°C. The plates were examined for the mycoflora under microscope for mycelial growth, sporulation, colour and pigmentation. The experiment was repeated to detect more number of mycoflora on the seed. Pathogenic fungi found in seeds were isolated into pure cultures and identified. Identification of fungi was made by using a standard key as described by Champion (1997); Marthur and Olga (2003) and Warharm (2008).Data collected were subjected to analysis of variance using STAT Graphic.5. Significant mean differences were determined with standard error of means.

## RESULTS AND DISCUSSIONS

Eight and eleven seed mycoflora of soybean were isolated respectively by Blotter technique and agar method, and identified from the seed samples varieties. The results presented in **table 1** below indicated that, the predominant fungi associated with seeds were *Aspergillusniger*, *Aspergillusflavus*, *Fusariumoxysporum*and *Rhizopusstolonifer*which were isolated from all varieties tested according to both methods.

However, there is also the presence of fungi such as *Macrophominaphaseolina, Fusariumculmoreum, Verticillium, Trichoderma*and others. The frequency of the occurrence of these organisms varied from different varieties. *M. phaseolina* was present in Goinia and absent in the others samples. *Trichodermasp* and *Gonatobotryssp* present on all varieties have been isolated on blotting paper. Furthermore, *Cercosporakikuchii*was present in all the varieties by the agar method.

Some previous work has shown similar results, Umechuruba and Nwachukwu (2002) and Goulart (1997) also detected 15-20 species of seed- borne mycoflora which occurred on soybean seeds *viz., Phomopsis*spp., *Colletotrichumtruncatum, Cercosporakikuchi, C. sojina, Rhizoctoniasolani, Sclerotiniasclerotiorum, Fusariumsemitectum, Aspergillus*spp. and *Pencillium*spp. Solanke et al. (1997) isolated *Aspergillusniger, A. flavus, Fusariummoniliforma, Curvularialunata, Alternariaalternata*and *Pencillium*spp. from soybean cv. PK-472 and MACS-13 seeds by agar plate, blotter paper and moist sand methods. The results were also in agreement with the findings of Agarwal et al. (2006) and Shovanet al. (2008).

It has been reported that infected seed can provide primary inoculums for infestation of new crop and seed borne pathogens may be dispersed for long distances with it (Hartman et al.,1999). This list indicated that the pathogens causing severe seedborne diseases on soybean are carried through the seed. Mostof them are common fungi, some of which get associated in the field and some in storage depending upon the nature of storagepracticed. The work of Rizvi and Yang, (1996) highlighted the presence of *Fusarium* spp.and*R. solani* from some seedlings. Similarly, the association of *R. solani* and *F. oxysporum* in causing root rot of soybean in Illinois has been reported by Datnoff and Sinclair (1988).

**Field Performance**

Average performances of thesoybean are shown in **Table 2.** Lift rates of different varietiesranged from 85 to 95%. TGX1835-10E presented the high rate with 95.08% followed by the others varieties.

Mean values for plant height varied between 61.68 and54.21 cm with variety TGX 1835-10E producing the tallest plants followed by EMGOPA301 with 57.35 cm and GOINA with 54.21cm.

Average values for pods number per plant ranged from 48 to 42 pods. GOINA variety presented the high number of pods per plant with 48.6. However no significant difference was observed between these three varieties. In contrast, the previous work of Adeniyan and Ayoola (2007), Aduloju et al. (2009) in Nigeria have shown significant differences between the numbers of pods per plant of the soybean varieties used.

Averagegrain yields ranged from 802.2 to 4010.05 kg/ha.VarietyTGX1835-10E produced the highest grain yields while GOINA and EMGOPA301 had the least.

Average values of weight of 1000 grains varied from 139.6 g to 89.93 g. The TGX-1835-10E had a weight of 1000 seeds higher (139.6 g), followed by Goinia (102.2 g) and finally Emgopa 301 (89.93 g).

Rust infection on the leaves would have decreased photosynthetic leaf area which would have influenced the amount of carbohydrate necessary to fill ~~of~~ pods. This would explain the low weight of 1000 seeds of Goinia and Emgopa301varieties compared to Nzegang, (2011) which has the weight of 1000 seeds exceeding 110 g for the same varieties in North Cameroon.Significant differences in the weight of 1000 seeds and their yields between varieties were also reported in the work of Aduloju et al (2009); Adeniyan and Ayoola (2007; 2006).

Yields obtained in Northern Cameroon by Nzegang (2011) are higher than those we have achieved. He obtained yields of 4443 and 2697 kg/ha respectively for GOINIA and EMGOPA301 varieties. These low yields obtained in this work would be linked to the negative effect of the rust on these varieties as the filling of pods and the formation of seeds were interrupted at early maturity. In fact, illness would have caused the malfunction of the plant.

There was no incidence and severity of rust infection in vegetative growth phase, however, in reproductive and maturity phases, incidence of rust infection rose to 100% for some varieties **table 3.** There were significant differences among the three soybean varieties in their reactions to the disease in there productive phase. Rust severity varied from 16.13 (TGX-1835-10E) to 60.55% (EMGOPA301) in reproductive phase. In maturity, Emgopa 301 and Goinia varieties have reached 100% severity while the TGX-1835-10E variety presented 34.30% severity.

The data obtained in this study revealed that rust infection is a serious problem in the environment under evaluation. However the TGX-1835-10-E variety proved resistant compared to the other two (Emgopa 301 and Goinia) who were susceptible to the disease.

High severity rate among exotic varieties would be due to the high temperature and humidity of environment which initially have extended their growing season and then facilitated the development of *Phakopsorapachyrhizi*. Twizeyimana et al. (2008) in Nigeria also showed that the variety TGX-1835-10-E was the most resistant to rust soybean among varieties studied.

The results obtained showed that there is a significant positive correlation between soybean grain yield and 1000-grain weight. Similarly, a significant negative correlation was observed between rust incidence and the weight of 1000 seeds as well as between rust incidence and grain yield.

## CONCLUSIONS

Results of this work show that the three varieties of soybean seed studied are infected by several fungal species which include *Aspergillusniger, Aspergillusflavus, Fusariumoxysporum*and *Rhizopusstolonifer*. Field evaluation revealed that rust infection is a serious problem in the locality where the experiment was conducted. The TGX-1835-10-E variety, which is already popularized in the locality proved to be the strongest with also a good yield compared to the others. Follow-up studies are recommended to evaluate the effect of phytosanitary treatments and off-season on these varieties. Similarly searching of biofungicides against this disease remains a challenge.

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## APPENDICIES

**Table 1: Predominant Mycoflora of Soybean Varieties Assayed By PDA and Blotter Methods**

| Fungalspecies | Blotter Technique | | | Agar Plate Method | | |
|---|---|---|---|---|---|---|
| | TGX-1835-10-E | Emgopa 301 | Goïnia | TGX-1835-10-E | Emgopa 301 | Goïnia |
| *Aspergillus flavus* | + | + | + | + | + | + |
| *Aspergillus niger* | + | + | + | + | + | + |
| *Cercosporakikuchii* | + | + | + | - | - | - |
| *Fusariumculmoreum* | - | - | - | - | - | + |
| *Fusariumoxysporum* | + | + | + | + | + | + |
| *Fusariumsubglutinans* | - | + | + | - | - | - |
| *Gonatobotryssp* | - | - | - | + | + | + |
| *Macrophominaphaseolina* | - | - | + | - | - | + |
| *Microdochiumsp* | - | - | - | + | - | + |
| *Rhizoctoniasolani* | - | - | - | - | + | + |
| *Rhizopusstolonifer* | + | + | + | + | + | + |
| *Trichodermasp* | - | - | - | + | + | + |
| *Verticiliumsp* | + | + | + | + | - | - |
| **Total** | 6 | 7 | 8 | 8 | 8 | 10 |

+ = Presence; - = Absence

**Table 2: Field Performance of Soybean Varieties**

| | Varieties | | |
|---|---|---|---|
| | TGX1835-10E | EMGOPA301 | GOINIA |
| **Lift rate** | 95.08[a] | 85.47[b] | 86.54[b] |
| **Plant height** (cm) | 61.68[a] | 57.35[b] | 54.21[c] |
| **Pods number /plant** | 46.8[a] | 42.46[a] | 48.6[a] |
| **Grain yield** (kg/ha) | 4010.05[a] | 802.35[b] | 870.86[b] |
| **Weight of 1000 grains** (g) | 139.6[a] | 89.93[c] | 102.2[b] |

Mean of triplicates values with different letters are significantly different ($p < 0.05$) for each line

**Table 3: Rust Severity and Incidence on Different Varieties**

| | Vegetative Phase | Reproductive Phase | Maturity |
|---|---|---|---|
| | | **Rust severity** | |
| TGX1835-10E | 0 | 16.13 | 34.30 |
| EMGOPA301 | **0** | **60.55** | 100 |
| GOINIA | 0 | 50.50 | **100** |
| | | **Rust incidence** | |
| TGX1835-10E | 0 | 80 | 100 |
| EMGOPA301 | 0 | 100 | 100 |
| GOINIA | 0 | 100 | 100 |